nature portfolio

Corresponding author(s): Melanie C. Dispenza, MD, PhD

Last updated by author(s): Mar 31, 2023

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Data were collected in part via: - REDCap - Epic electronic medical record - BD Accuri C6 Plus flow cytometer
Data analysis	Graphpad Prism 9.2.0 software was used for data analysis. No custom algorithms or code were utilized.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The data that support the findings of this study are not openly available, but are available from the corresponding author upon reasonable request. All requests

must be clearly described in writing. All shared data will be de-identified according to applicable regulations. Data are located in controlled access data storage within the Johns Hopkins University School of Medicine.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Food allergy affects men and women equally, and therefore this trial attempted to recruit equal numbers of male and female participants. Because the mechanisms of food allergy pathogenesis and acute reactions are identical between sexes, it is expected that the results herein would apply to men and women equally. For this trial, patients were asked to self-report their biologic sex (options included male and female) and gender identity (options included male, female, and unspecified/ prefer not to answer). Based on this self-reporting, patients enrolled in this trial demonstrated the expected gender ratio of male to female (50% each). One patient identified as a transgender male (assigned female sex at birth), thus 60% of the enrolled subjects were female sex. This study was not powered to perform sex-based analyses.
Population characteristics	Eligible patients were 18 years of age or older at screening with a history of an IgE-mediated allergy to peanut. Full eligibility criteria are listed in the Methods. Demographic data is depicted in Table 1. Their mean age was 28 years (range, 23 – 36 years); 6 were female sex (60%), 9 were Caucasian (90%), and 3 were Hispanic or Latino (30%). The age range in the trial was limited to adults, as children were not approved for enrollment under the FDA Investigational New Drug application.
Recruitment	Patients were recruited from the Johns Hopkins University Allergy and Clinical Immunology outpatient clinic and through IRB-approved advertising on social media. Patients who responded to advertisements were initially screened by telephone. If determined eligible, patients were remote consented prior to Visit 1 by teleconference in compliance with FDA 21 CFR Part 11 prior to their first study visit. Recruitment was in part biased based on social media usage (and therefore also likely biased to age group [18-45]). However, this age group was well within the target population for enrollment. Young adults and adolescents have a higher rate of accidental exposure and mortality as a result of food allergies than younger children, so there is an unmet need for therapies directed at food allergy, especially in this age group.
Ethics oversight	This trial protocol was approved by the Johns Hopkins University School of Medicine IRB (IRB00223615) and United States Food and Drug Administration (under Investigational New Drug application #142734).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was determined pre-trial based on the primary outcome, the change in patients' threshold dose of peanut to induce clinical reactivity while taking acalabrutinib as compared to baseline. We estimated that 10 subjects allowed for 80% power to detect a 3-fold increase (1.1 natural log units; i.e. 1 food dose escalation) in the threshold food dose using a paired t test with $p<0.05$ as an approximation. For this sample size determination, the primary endpoint was assumed to be normally distributed with a standard deviation of 1.1 natural log units.
Data exclusions	No collected data were excluded from analyses. All patients who received acalabrutinib (n = 10) were included in the data analyses. One blood sample was misplaced by the core pathology laboratory, resulting in missing data for Patient 006's complete blood counts at Visit 2; this was considered to have been a random occurrence, and sampling could not be repeated based on the timing of the sample draw. Basophil activation data was lost for Patient 008's Visit 3 due to cytometer malfunction; this was also considered to have been a random occurrence. Due to the randomness of these 2 individual events, no statistical adjustments were made.
Replication	Data were collected using standardized testing and/or procedures whenever possible to ensure reproducibility of findings. Oral food challenges and skin puncture testing were performed using procedure protocols and interpretation as recommended by society guidelines. Symptoms and clinical reactivity during oral food challenges were assessed using a published standard scoring system as described in the Methods. Laboratory testing was performed in CLIA certified clinical laboratories.
Randomization	None (single-arm study)
Blinding	None (single-arm study)

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT05038904
Study protocol	The full trial protocol and statistical analysis plan are included in Supplemental information, along with summaries of modifications that were made to the protocol and statistical analysis plan over time.
Data collection	All study procedures were conducted in the Clinical Research Unit at the Johns Hopkins University Bayview Campus in Baltimore, Maryland after informed consent was obtained. All study visits occurred between December 2021 and October 2022.
Outcomes	The predetermined primary endpoint was the change in patients' threshold dose of ingested peanut protein to elicit an objective clinical reaction during food challenge after acalabrutinib pretreatment compared to patients' baseline, as assessed by symptoms during oral food challenge using a modified PRACTALL scale to score symptoms. A key secondary endpoint included the change in the severity of clinical reactions during oral food challenge. Other secondary endpoints included size of the skin test wheal to peanut extract and the percent of basophils activated ex vivo by peanut extract while receiving acalabrutinib compared to baseline. Safety endpoints included electrocardiography and laboratory blood testing, including complete blood counts and differentials, serum chemistries, and liver function tests. Exploratory endpoints included changes in circulating quantitative immunoglobulins and serum specific IgE to peanut and peanut components.

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Whole blood samples were drawn into 4 mL lithium heparin phlebotomy tubes (BD Biosciences) and inverted 8-10 times. Whole blood was then incubated with mouse IgM anti-human-IgE monoclonal antibody (clone 6061P, Hybridoma Labs), the indicated dilutions of peanut extract (Greer), 1 µM N-formylmethionyl-leucyl-phenylalanine (Sigma), or vehicle (Greer incipient control solution) in PAGCM buffer (piperazine-N,N′-bis[2-ethanesulfonic acid] + bovine serum albumin [MP Biomedicals] + glucose [Sigma-Aldrich] + 1.7 mM calcium + 1.7 mM magnesium) for 30 minutes at 37 °C. Cells were then fixed using Phosflow Fix Buffer (BD Biosciences), centrifuged at 400 x g for 5 minutes, and resuspended in Pipes buffer with 1 mM EDTA and 0.25% bovine serum albumin. Cells were blocked with 1 mg/mL nonspecific human IgG (MB Biological) and then incubated with the fluorescently-conjugated monoclonal antibodies anti-CD63 (1/1000, BD-Pharmingen) and anti-FcεRIα (clone CRA-1, 1/250, Life Technologies) for 25 minutes at room temperature, then with secondary antibodies anti-CD123-PE (1/100, BD Biosciences), anti-mouse IgG2b-AlexaFluor488 (1/1000, Life Technologies), and anti-mouse IgG1-AlexaFluor647 (1/1000, Life Technologies) for 25 minutes at room temperature before analysis.
Instrument	BD Accuri C6 Plus flow cytometer

Software	Data were analyzed on BD Accuri C6 software. No custom code was utilized.
Cell population abundance	Sequential gating was used to ensure that the correct population was analyzed. Basophils comprised of roughly 0.1-1% of peripheral blood mononuclear cells as was expected (with a range of 11 to 102 thousand basophils per mm3 of blood). Post-sort fractions were not analyzed.
Gating strategy	Cells in whole blood were first gated on FSC-A vs SSC-A on peripheral blood mononuclear cells. Cells were then gated on CD123+ FcERIa+ cells. The percentage of CD63 positive (high) cells was recorded for each sample.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.